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NITROGEN EXCRETION BY THE SPINY LOBSTER *JASUS EDWARDSI* (HUTTON): THE ROLE OF THE ANTENNAL GLAND

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It is now evident that the antennal gland functions to a greater or lesser extent as an osmotic and ionic regulator in both freshwater and marine Crustacea (Beadle, 1957; Robertson, 1957; Shaw, 1960; Lockwood, 1962; Potts and Parry, 1964). Furthermore, there is a considerable amount of evidence to show that reabsorption, particularly of electrolytes and potential metabolites such as glucose, and secretion may be involved in the process of urine formation as fluid passes through the antennal gland (Martin, 1957, 1958; Parry, 1960; Kirschner, 1967).

Because it produces a urine, is concerned with salt and water regulation, metabolite reabsorption, and has the power to secrete certain molecules, there is a striking resemblance between the functions of the antennal gland of Crustacea and the mammalian kidney. The two organs may therefore be regarded as being physiologically analogous in some respects. However, it would be imprudent to ascribe to the antennal gland all the functions of the mammalian kidney, and to think of it as a true excretory organ, that is, one concerned particularly with the elimination from the animal of nitrogenous waste products of protein metabolism. Whether the antennal gland does play an important part in the excretion of nitrogenous waste products is still in doubt.

Determinations of nitrogen excretion by Crustacea are relatively few. Figures are available for rates of nitrogen excretion by whole animals, without reference to the contribution of urine nitrogen (Dresel and Moyle, 1950; Needham, 1957; Sharma, 1966), or conversely, for urine nitrogenous components with no determinations of their significance to the general problem of nitrogen excretion (Delaunay, 1931). Although it is possible, using the data available, to make a rough estimate of the contribution of urinary nitrogen to overall nitrogen excretion, there has been no specific attempt to relate these two factors directly. This omission is perhaps surprising, and represents a real gap in our knowledge of antennal gland function in Crustacea.

The aim of this study was to determine the role of the antennal gland of *Jasus edwardsi* in relation to general nitrogen excretion by this animal. Nitrogen loss from whole animals was measured and the contribution of urinary nitrogen to total nitrogen excretion was estimated by determining various nitrogenous components in the urine and the rate of urine flow. In this way it was intended to define, more accurately than current information allows, the relative importance of the antennal gland as an excretory organ.

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Materials and Methods

Animals were collected at Kaikoura, on the North Canterbury coast, and transported immediately to the laboratory in Christchurch. They were kept in a large concrete tank supplied with aerated, circulating sea water maintained at a temperature of $14 \pm 0.5°$ C. Animals were used within a week of being collected, and were not fed while in the holding tank or during experiments.

Total nitrogen (TN) and ammonia nitrogen (AN) excretion were determined by analyzing small volumes of sea water in which an animal had been living. The experimental animal, after being dried as thoroughly as possible, was weighed and then left overnight in a large glass desiccator containing 1500–2000 ml of filtered sea water (Millepore, mean pore diameter 0.45 μ). The desiccator lid, sealed with Vaseline and clamped in place, was closed at the top with a rubber bung which contained an air inlet into the sea water and also a shorter outlet.

Jasus was found to be extremely sensitive to oxygen lack, and continuous aeration of the sea water was necessary. A current of air was passed first through a large boiling tube containing 1 N sulfuric acid to remove any ammonia, then through a second tube containing distilled water before passing into the sea water in the experimental chamber. Air leaving the chamber passed through two further tubes, each containing 50 ml of 0.01 N hydrochloric acid.

AN in the bathing medium was determined by direct steam distillation of 10 ml samples of the sea water in a Markham unit, after the addition of 10 N sodium hydroxide solution, and subsequent titration of the distillate against 0.01 N hydrochloric acid. TN was determined by the Kjeldahl method. Any ammonia which may have passed with the current of air from the bathing medium was carried over into the two tubes containing dilute acid. This nitrogen fraction, determined by titration of the acid against a dilute standard solution of sodium hydroxide, was taken into account when rates of excretion of TN and AN were calculated.

Urine samples were obtained as follows. The areas around the nephropores were dried, and the anterior openings of the gill chambers blocked with small wads of filter paper. The membrane covering the openings from the bladder appeared to be extremely irritable; slight pressure on the membrane with the tip of a glass micropipette was usually sufficient to produce a flow of urine which was then drawn into the pipette by suction. In some cases it was necessary to push the tip of the pipette through the opening in the membrane to obtain a urine sample. This was done with extreme care to prevent any tearing of tissues, and urine obviously contaminated with blood or tissue fragments was discarded.

The total nitrogen content of urine was determined by the Kjeldahl method. Ammonia and urea in urine were determined by the microdiffusion method of Conway (1950), urea being estimated after treatment of aliquots of urine with powdered urease in an acetate buffer. Amino compounds in the urine were determined by the method of Rosen (1957), using a Bausch and Lomb Spectronic 20 colorimeter. Concentrations of amino compounds were related to glycine standards which were used throughout.

To estimate quantitatively the contribution of urinary nitrogen to general nitrogen excretion, it was necessary to determine urine production rates of animals. The openings from both antennal glands were blocked with dental cement. Animals were then weighed, placed in sea water and re-weighed after a period of 6 to 8

hours. Rates of urine flow were calculated on the assumption that weight increase was due to the accumulation in the bladder of urine which would normally have been voided.

Duplicate analyses were made for all determinations of the various nitrogen components in both bathing medium and urine samples.

The composition of body fluids and the rate of urine flow of some Crustacea may be affected by experimental stress or excessive handling (Riegel, 1960; Riegel and Kirschner, 1960). In the present study, all chemical analyses and determinations of urine flow rates were made using animals which had not previously been used for experiments. Animals could therefore be regarded as being as near 'normal' as possible when measurements were made.

Results

The rates of excretion of TN and AN into the surrounding medium, and AN as a percentage of TN loss are shown in Table I.

Table I

Rates of excretion of total nitrogen and ammonia nitrogen by Jasus edwardsi

Animal no.	Total nitrogen (TN) (μg N/hr/g body wt)	Ammonia nitrogen (AN) (μg N/hr/g body wt)	$\frac{AN}{TN} \times 100$
1	4.0	3.2	80.0
2	2.1	1.8	85.7
3	3.8	2.7	71.1
4	4.1	2.7	65.8
5	1.7	1.2	70.6
6	1.0	0.6	60.0
7	8.8	—	—
Mean ± S.D.	3.6 ± 2.4	2.0 ± 0.9	72.2 ± 8.5

TN excretion rates varied over a considerable range, the fastest rate being almost nine times greater than the slowest rate recorded. However, at all rates of excretion AN represented a consistently high percentage of TN loss, with a mean value of 72.2% TN and a range of 60.0% to 85.7%. It is considered that this figure represents as accurate an estimate of AN as possible under the conditions of these experiments. Bacterial decomposition of nitrogenous components other than ammonia in the bathing medium could conceivably increase the recorded AN/TN ratio above its true value. Because of this possibility, sea water was initially made bacteria-free by filtration and animals were kept in the experimental chamber for only a short time, to ensure that changes in the ammonia content of the sea water due to the action of bacteria were minimal.

Concentrations of the four nitrogenous urine constituents measured are shown in Table II.

Of the three specific components determined, urea represented the largest fraction. However, urea, ammonia and amino compounds together represented only 21.2% of total urine nitrogen. By far the greatest proportion of urine nitrogen was due to constituents other than these three common excretory products.

TABLE II

Concentration of total nitrogen, ammonia, urea and amino compounds in the urine of Jasus edwardsi

	Urine nitrogenous constituents				
	Total urine nitrogen	Ammonia	Urea	Amino compounds	Unidentified (by substraction)
Mean conc. (mg. %)	21.2	1.5	4.7	5.7*	
S.D.	±4.1	±0.7	±1.8	±3.1	
Mean conc. (μg N/ml)	212	12.6	21.8	10.6	167
% total urine nitrogen	—	5.9	10.3	5.0	78.8
No. of observations	7	7	6	6	

* Corrected for urine ammonia and expressed as equivalent to glycine concentration.

The determination of urine flow rates in aquatic animals is a difficult problem, and the method used in this study is open to various criticisms. Primary urine is probably produced by ultrafiltration of the blood into the antennal gland of Crustacea (Kirschner, 1967). In blockage experiments it is possible that the accumulation of large volumes of urine in the bladder could produce a back pressure sufficient to reduce the rate of urine flow. For this reason, animals were re-weighed a relatively short time after the urinary openings were blocked, so that the flow of urine through the antennal gland itself was unimpeded.

Rates of urine production in Table III are remarkably consistent, and it is unlikely that this degree of consistency would have been achieved if the weight increases observed were due to factors other than the accumulation of urine, such as the swallowing of sea water by experimental animals.

The assessment of the contribution of the nitrogenous constituents in the urine of *Jasus* to overall nitrogen excretion by the animal is shown in Table IV. The percentage contribution of urine nitrogen fractions to TN and AN excretion was assessed using the mean values for nitrogenous excretion rates, urine constituents and flow rates. In calculating the volume of urine produced per unit time it was

TABLE III

Rates of urine production in Jasus edwardsi

Animal no.	Body weight (g)	Urine production rate (% body weight/day)
15	166.9	4.3
16	165.8	3.1
17	198.3	6.5
18	178.3	3.8
19	122.8	4.8
20	244.6	6.2
	Mean	4.8
	S.D.	±1.1

TABLE IV

Contribution of nitrogenous constituents in the urine of Jasus edwardsi *to total nitrogen and ammonia nitrogen excreted*

Urine nitrogenous constituent	% contribution to total nitrogen excreted	% contribution to total ammonia excreted
Ammonia	0.7	1.3
Urea	1.2	—
Amino compounds	0.6	—
Unidentified	9.1	—
Total nitrogen	11.6	—

assumed that the urine had the same specific gravity as the sea water used in experiments which, at room temperature, was 1.022.

Nitrogen loss in the urine would account for 11.6% of TN excreted. However, the contribution of the common excretory products ammonia, urea and amino nitrogen would be only 2.5% of TN excreted. The large unidentified nitrogen fraction, presumably itself made up of several components, would represent 9.1% of TN excreted. The main excretory product of *Jasus* is ammonia (Table I), yet urine ammonia would contribute less than 1% of TN loss, and only 1.3% of AN excreted by the animal.

DISCUSSION

The general pattern of nitrogen excretion in *Jasus* is typical of that shown by a large variety of aquatic invertebrates (Prosser and Brown, 1961), in that the principal excretory product of the animal is ammonia. This compound represented just over 70% of TN excreted by *Jasus*, and equally high AN/TN ratios have been recorded for other aquatic Crustacea (for review see Parry, 1960). In addition to this qualitative similarity, it is clear from Table V that the amount of nitrogen excreted by *Jasus* lies within the range of excretion rates per unit body weight measured for other aquatic Crustacea.

TABLE V

Nitrogen excretion rates in some aquatic crustaceans

Species	Rates of nitrogen excretion (mg N/10 g body wt/day)	Reference
	Total nitrogen	
Jasus edwardsi	0.9	This paper
Carcinus maenas	0.4	Needham (1957)
Gammarus locusta	4.9	Dresel & Moyle (1950)
G. zaddachi	6.0	Dresel & Moyle (1950)
G. pulex	2.3	Dresel & Moyle (1950)
Marinogammarus marinus	1.1	Dresel & Moyle (1950)
M. pirloti	2.9	Dresel & Moyle (1950)
	Ammonia and urea only	
Orconectes rusticus	3.5	Sharma (1966)

Although there appears to be some variation in the rates of nitrogen excretion between different species, some standardization of experimental procedures will be necessary before it can be established whether or not these variations indicate real differences in excretion rates. Rates of nitrogen excretion for a single species may vary over a considerable range depending on such factors as the nutritional state of animals, the presence or absence of other animals, stage in molt cycle or the volume of sea water in which an animal is living (Needham, 1956, 1957).

Delaunay (1931) found that the concentrations of ammonia, urea, uric acid and amino nitrogen in the urine of *Maja* were extremely low, and concluded that in this animal the antennal gland was not primarily concerned with nitrogen excretion. Similarly, Parry (1960) reviewed work on antennal gland function in Crustacea, and considered that the information available showed conclusively only that the antennal gland of marine Crustacea was important as an ionic regulator, and that its role in general nitrogen excretion was likely to be of little consequence.

The present study is the first direct attempt to quantify the role of the antennal gland in relation to total nitrogen excretion. The common excretory products measured in the urine would contribute an extremely low proportion of TN and AN excreted by *Jasus*. Almost 90% of all soluble nitrogen excreted is non-urinary in origin. The waste nitrogen is mainly in the form of ammonia, and presumably much of it leaves the animal by diffusion through highly permeable surfaces such as the gills. Therefore, in terms of overall nitrogen loss from *Jasus*, urine nitrogen is of very minor importance.

Despite this conclusion, the antennal gland may be concerned with the excretion of materials which cannot be disposed of by simple diffusion. Most of the nitrogen in the urine of *Jasus* was not identified, and this unknown fraction would contribute almost 10% of TN excreted by the animal. Equally large amounts of unidentified nitrogen in the urine of other Crustacea are simply a reflection of the very limited range of analyses which have been carried out on urine samples. Secretory activity is widespread in various parts of the antennal glands of Crustacea (Lison, 1942). Ramsay (1961) suggested that secretion may be of importance in eliminating complex nitrogenous compounds which are either too big or otherwise unsuitable to enter the antennal gland by filtration, and that secreted materials could account for at least some of the nitrogen hitherto unidentified in the urine of Crustacea.

Secretion/digestion systems of the type known to be present in the antennal gland of the freshwater crayfish (Riegel, 1966) are likely to be present in the antennal glands of other Crustacea. Such systems may be important in the elimination of complex waste products, and further detailed analysis of the urine of Crustacea may reveal that the antennal gland does have a true excretory function, although it appears that the organ does not have a quantitatively important role in overall nitrogen excretion.

Summary

1. Nitrogen excretion by *Jasus edwardsi* has been investigated.
2. Ammonia nitrogen accounted for 72% of total nitrogen excreted.
3. Urine production rate was 4.8% of the body weight per day, and urine nitrogen would contribute 11.6% of the total nitrogen loss from the animal.

4. Ammonia, urea and amino compounds represented 21.2% of total urine nitrogen. These compounds together accounted for 2.5% of total nitrogen excreted, and urine ammonia for 1.3% of total ammonia loss.

5. Most nitrogen excreted is non-urinary in origin, and it is concluded that the antennal gland of *Jasus* is not important as far as total nitrogen loss from the animal is concerned.

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